Sam Seifter in His Own Words

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The following is a dictated, unedited, partial account of my scientific life.

I was born in Cleveland, Ohio and received a wonderful education in its public schools. This experience applied to all fields of learning, but especially to natural history and the physical sciences. In high school, I took competitive state-wide and national examinations in various fields, and won a joint first place scholarship in history to Oberlin College. I also won general scholarships to various state universities in Ohio. Because I graduated from high school in 1933, in the depth of the Great Depression, I could not afford, even with the scholarships, to attend any of the colleges. A humorous side effect was that Oberlin, which had one of the worst football teams in the country, offered me a supplemental football scholarship to make my attendance possible, although I had not even played high school football. I wisely turned down the offer.

I remained out of school for almost three years. For a year-and-a-half of that time I walked the streets of Cleveland looking for a job, when almost a fifth of the population of the United States was unemployed. I finally found a job in a small clothing factory where I worked a twelve-hour day for the handsome wage of eight dollars a week. Each day I spent the morning as secretary and typist, the afternoon at the back-breaking task of pre-shrinking heavy bolts of cloth to be made into suits, and the evening delivering the finished suits (on public buses) to neighborhood clothing stores all over the city. Needless to say I spent my free time (!) organizing workers in the shop into the Amalgamated Clothing Workers of America. That landed me with both feet in the great Ohio steel strike of 1936, but that is another story.

In that year, I was finally able to enroll as a student at Ohio State University, majoring in chemistry. Because of the studying I had done at home, I made up almost a year in proficiency credits, including those in English and chemistry. My science experiences started early with my brothers Eli and Joe, who later also had academic careers in chemistry and pharmacology. Amusingly, when I was eight years old, I discovered how to make the equivalent of Sterno, using the finely powdered soot from a coal furnace and appropriate amounts of beef fat or candle wax. In other experiments I collected hundreds of flying grasshoppers (*locusts: Schistocerca*) and tried to convert them from herbivores to carnivores by a process of force feeding of housefly larvae by manipulation of the grasshopper mandibles. At the age of fifteen, in my chemistry class, I wrote theoretical papers developing a system for determining the strength of acids by "coefficients of acidity," equivalent to a system, then new, of the normality of acids and bases. On paper I also developed a process for making synthetic rubber from unsaturated hydrocarbons. In 1935 my brother Joe, by then a member of the chemistry faculty at the University of Oklahoma, presented my work on the reduction of methemoglobin to hemoglobin at a meeting of the American Chemical Society in Kansas City. The abstract became my first publication. I also did work at home on the isomeric forms of copper ammonium picrates; and on the effects of lead poisoning on the fertility of male and female rats. I should also mention that I invented a process for making instant tea by low temperature distillation. The tea could be flavored with lemon distillate or cherry distillate to satisfy the palates of Russian or Rumanian tea drinkers. I made no money from these ventures, but they served to introduce me to my wife – another story.

My first full publication was in 1938, in my junior year at Ohio State. It described experimental work on rare earth separations achieved by crystallization procedures. This work was published in *Analytical Chemistry* and later almost got me into a group at the Manhattan Project that isolated plutonium. (One of my childhood friends in Cleveland was Jack Gofman who did work with Glenn Seaborg on the isolation of plutonium. That is the same Jack Gofman of the Lawrence Radiation Laboratories who went on to do the first work, by use of centrifugal flotation, on plasma lipoproteins.)

At Ohio State, I also prepared carbohydrate intermediates for Melvin Wolfram, a pioneer in carbohydrate chemistry and the structure of heparin.

During the summer vacations in my undergraduate years, I worked for Dr. Torald Sollmann, Chairman of Pharmacology and Dean of the Western Reserve College of Medicine. I did analytical work on the bismuth and arsenic compounds then used in the treatment of syphilis. I also worked with him on the references for his famous Textbook of Pharmacology. With my brother Joe, then Assistant Professor of Pharmacology, I helped synthesize the very toxic metal alkyls of bismuth, arse-

nic, zinc and mercury. When I went back to Ohio State, for my honors in chemistry, I then tried to synthesize the alkyls of the rare earth elements, compounds which at that time were thought to be impossible of existing. In fact, I failed in the synthesis. At that time, Fermi and his colleagues in Italy and Hahn and Meitner in Germany were working on the transuranium elements. I was assigned to translate their papers, and to help in a project on the isolation of the products of cyclotron bombardment of rare earth elements. The only cyclotron in the Midwest in the 1930s was at the University of Michigan, so we took our materials to Ann Arbor, had them bombarded and rushed back to Columbus, Ohio, in a Ford flivver, to analyze the elements formed. That was a replay of the traditional Michigan-Ohio State football rivalry.

My work on the rare earths also included the preparation of acetylacetonates and, with the help of the X-ray department, we obtained powder diagrams of the compounds.

Following my graduation with honors in chemistry, in 1939, with the help of Dr. Sollmann, I was admitted to the Graduate School at Western Reserve University. With funds from the Commonwealth Foundation, I prepared to do my dissertation on the chemistry of complement with Louis Pillemer and Enrique Ecker. While I was a student, I gave lectures in the medical school biochemistry course; the lectures dealt with the newly evolving field of protein chemistry, as exemplified by the work of Cohn, Edsall and Oncley. In the immunology course, I lectured on the nature of antigens and antibodies. In 1944 I became instructor of immunology and in 1945 senior instructor in immunochemistry.

Covering the period of my dissertation work, I published, with Pillemer and others, almost twenty papers on the chemical nature and function of complement components, especially as they operated in what is now known as the classical pathway of complement action. We also defined some of the ways in which separated, partially purified components interacted in the pathway. I then studied, in the light of the new knowledge, how complement components were affected in various human diseases and especially I did work on complement in the nephrotic syndrome and in lupus erythematosus. As a side issue, we showed that complement components could be excreted in proteinuria, an early demonstration of the loss to the urine of specific functional proteins (other than albumin).

One of the treasured highlights of my years at Western Reserve University occurred at the AAAS meeting in the winter of 1944. Oswald Avery presented his work on DNA transformation regarding pneumococcal polysaccharides. At the same meeting, I followed him with a paper describing our work on complement and disease. We both received extensive write-ups in the Sunday *New York Times* and in *Time* magazine. You can understand what a humbling experience this was for me when his work became one of the seminal discoveries of modern biology and mine ended in a whimper.

During the war years, I worked on the preparation of human plasma for field transfusion and I also worked on the clinical use of early preparations of penicillin.

In 1945, I moved to Brooklyn to the Long Island College of Medicine as assistant professor of biochemistry. That school was one of the few that admitted large numbers of Jewish and Catholic students, many of whom were as outstanding as students who wound up at Harvard and Yale. The school later became the Downstate Medical School of the University of the State of New York. I continued to teach, giving as many as ninety lectures a year in all fields of biochemistry, including structure, metabolism, endocrinology and nutrition. I also supervised laboratories and gave many lectures on laboratory methods. The fields of protein chemistry, intermediary metabolism, hormones and vitamins were bursting with new knowledge that is the backbone of clinical medicine today. I can remember running from Federation meetings to the classroom to present for the first time such subjects as the pentose phosphate pathway, the citric acid cycle, the nature of CoA, and the structure and functions of adrenal corticosteroids.

My research in those years centered on coenzymes and metabolism and the effects of dietary protein deficiency. We were on the road to demonstrating the participation of molybdate in xanthine oxidase activity (having just published, in *Analytical Chemistry*, a method for determination of molybdenum as molybdate) when Westerfeld came out with the definitive paper on the presence of molybdate in that enzyme. Our consolation prize was that the U.S. Bureau of Mines, for the next ten years, used our method for the determination of molybdate in ores!

In 1951, we published a paper on the determination of glucose and glycogen using the anthrone reagent. The paper became a citation classic, notable for at least two other findings. It demonstrated for the first time that hemoglobin had a "glucose equivalent;" however, it did not relate the glucose to hemoglobin A1C, a marker shown later to be important in following the effectiveness of diabetic treatment. The paper also showed that gelatin, derived from collagen, reacted with the anthrone reagent, allowing the inference that collagen is a glycoprotein.

In 1954, as a result of political disagreements with the Downstate Medical School, I left that institution and through the intervention of Drs. Zimmerman and Laszlo at Montefiore Hospital, I went to the newly built Long Island Jewish Hospital, where I set up and ran the clinical chemical laboratory for almost two years.

In the same year, when the Albert Einstein College of Medicine was being planned, Dr. Abraham White agreed to appoint me to the staff of the Department of Biochemistry, to which I moved when the school opened in 1955. With Dr. Hoberman (who was the first faculty appointment) and with Drs. White and Lauson, I helped plan a joint course in biochemistry and physiology and wrote, with Nancy Buckley, the first laboratory manual for the course.

Meanwhile, at the Long Island Jewish Hospital, I teamed up with Dr. Paul Gallop and formed a scientific association and personal friendship that lasted until his death a few years ago. We decided to work on collagen, focusing on its primary structure as determined by the isolation and characterization of peptides obtained by cleavage with the collagenase of *Clostridium histolyticum*. We purified the enzyme, established its physical parameters and developed assays based on viscometric, kinetic and colorometric features. We determined that the enzyme is a zinc metalloproteinase that required calcium ions for activation. These features of the bacterial collagenase were found by others to define mammalian zinc metalloproteinases, a group of enzymes now being actively pursued in the processes of development and of metastasis of tumors. We established the specificity of the enzyme as it acts on a collagen substrate. Later, with the cooperation of Carl Franzblau, Olga Blumenfeld, Elvin Harper, Mercedes Paz, Marcos Rojkind, Shizuko Takahashi, Hilda Carnicero and others, we described many of the covalent features of collagen, including the nature of aldehyde-derived crosslinks, the nature of the binding of glucose and galactose to hydroxylysine residues and some features of the glycosylases involved. We also studied the nature of subunits of collagen and, with Peter von Hippel and William Harrington, described some definitive physical features of the protein, especially the process of collagenolytic degradation. In a special side study, Thomas Morione, Anthony Robbins and I studied the accumulation of collagen in the pregnant human uterus and the removal of that collagen enzymatically fourteen days post-partum.

In those studies we developed many new methods, including: (1) the determination of aldehydes in collagen and elastin; (2) the absorption, at low temperatures, of collagenases to insoluble collagen, a property that led us to a method for purification of collagenase that was probably the first use of affinity chromatography for the purification of an enzyme (a method that had long been used in immunology); (3) probably the first use of galactose oxidase to establish the presence of galactose in glycoproteins. (Mary Jane Osborne had already used that enzyme to study galactose in lipids.); (4) the use of ammonia, hydrazine and hydroxylamine for nucleophilic cleavage of proteins, especially collagen, leading to analytical use of the Hoffmann, Curtius and Lossen rearrangements; and (5) from this the development of a method by Carl Franzblau, using hydroxylamine, for the determination of carboxyl groups in proteins.

Following the work of Partridge and Franzblau on the crosslinks of elastin, we studied certain aspects of the chemistry of desmosines and isodesmosines. We also studied the immunogenicity of collagens and described some of the antigenic groups responsible for generation of collagen antibodies.

In the field of carbohydrate biochemistry, in addition to our work on the anthrone method and the determination of glucose and galactose in various collagens, I worked with Nathan Sharon on establishing that lysozyme catalyzes a transglycosidation reaction; that last study allowed the preparation of intermediates that were used in X-ray studies of lysozyme action.

We also did studies, particularly with George Wu and Yoichi Urushizaki, on various aspects of hydroxylysine reactions. We showed that trypsin, in addition to cleaving peptides containing lysine or arginine residues, could cleave peptides containing hydroxylysine residues, extending the known specificity of trypsin. We also showed that the presence of galactose and glucose on hydroxylysine residues blocked that action of trypsin. We did chemical studies on the synthesis of proline from free hydroxylysine. Finally, we showed that smooth muscle cells in culture could produce phosphohydroxylysine residues in small amounts, and this required ATP. In the course of those last studies, we established methods for the detection of phosphohydroxylysine residues in proteins.

In studies of aging, we characterized many features of collagen production in cells in culture and, with the late Tom Robinson, Mahboubeh Eghbali, Bea Wittenberg, Maria Zeydel and Olga Blumenfeld showed the collagenous nature of struts in cardiomyocytes. We also studied the collagens and elastin of the hearts of rats as they developed from birth and as they aged.

With Shizuko Takahashi we studied the gamma glutamyl cycle in cells growing in culture, and developed a method using concanavalin A for purifying gamma glutamyl transpeptidase. With Song Han we published preliminary experiments on the recombinant production of bacterial collagenase. We also established methods for determining the types of collagen by use of collagenase.

With Marie Daly we established the nature of uptake of creatine by muscle cells in culture.

With Irwin Arias, Lawrence Gartner and Eric Bloch, we studied human milk, and isolated and characterized a steroid that appeared to inhibit the enzyme that puts glucuronyl groups on bilirubin.

With Anthony Tucci we made a detailed study of parot-

id gland butyrylcholinesterase, and showed the presence of amino acid residues at positions corresponding to those of hydroxyproline and hydroxylysine. That heralded the later finding by others that collagen-like segments occur in acetylcholinestrase. Later with Israel Silman and Mahboubeh Eghbali, we published a histochemical method for acetylcholinesterase based on a reaction with antibodies to collagen.

Berta Scharrer, Elvin Harper and I did an interesting study on fibers in cockroach nervous systems, showing by chemical means that they were collagen fibers. We also showed the existence of methionine sulfoxide in proteins of those tissues.

With Michael Dunn and Penny Haight we did extensive studies of proline metabolism in experimental schistosomiasis.

With Sara Rogozinski and Olga Blumenfeld we studied the glycation of collagen.

I should mention that among other publications, I wrote, with Sasha Englard, extensive reviews on subjects such as the energy metabolism of the liver, carbohydrate metabolism in diabetes and the biochemical reactions of ascorbic acid. We also wrote chapters on methods for purifying proteins and for detecting post-translational modifications of amino acid residues in proteins.

At the present, I am writing some historical papers dealing with the composer Borodin and his work as a chemist and especially on his relationship with Cannizzaro. I am writing about crosscurrents in the lives of Chaim Weizmann and Fritz Haber. I am also writing an article on possible evidence that syphilis occurred in Europe before the voyages of Columbus.